

Impact of early infant growth, duration of breastfeeding and maternal factors on total body fat mass and visceral fat at 3 and 6 months of age

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Short title: Determinants of body composition in early life

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Abstract

Background: Accelerated gain in fat mass in the first months of life is considered to be a risk factor for adult diseases, given tracking of infancy fat mass into adulthood. Our objective was to assess the influence of early growth, type of feeding and maternal variables on fat mass in early life.

Methods: In 300 healthy term infants we longitudinally measured fat mass percentage(FM%) by air-displacement-plethysmography at 1, 3 and 6 months and abdominal visceral and subcutaneous fat, measured by ultrasound, at 3 and 6 months.

Results: Both gain in FM% and weight-for-length in the first 3 months were positively associated with FM% at 6 months of age and visceral fat at 3 months of age. Gain in FM% and weight-for-length between 3 and 6 months was positively associated with visceral fat at 6 months. Breastfeeding duration associated positively with subcutaneous fat, but not visceral fat at 3 and 6 months. Maternal characteristics did not associate with FM% or visceral fat at 3 or 6 months.

Conclusion: Higher gain in FM% or in weight-for-length in the first postnatal months leads not only to higher FM%, but also more visceral fat. Exclusive breastfeeding appears to promote subcutaneous but not visceral fat in the first 6 months.

Introduction

The first three months of life are known to be a critical window for the programming of adiposity and cardiovascular diseases [1-3]. Unraveling the modifiable determinants that influence the adiposity and fat mass development in early life can provide valuable insights to support an optimal infant development. We have previously shown that the risk for type 2 diabetes and cardiovascular diseases at the age of 21 years differs according to weight-for-length gain during the first months of life, indicating that early growth might be a determinant for later life metabolic health [4]. One of the key elements driving early life growth is the nutrition provided to the young infant. Exclusive breastfeeding is the preferred feeding for newborn infants, being associated with a.o. less infections, better cognitive development, but also with a lower incidence of childhood obesity and type 1 and type 2 diabetes [5]. However, outcomes of studies on the influence of breastfeeding on infant's body composition are not straight-forward [6-9].

Apart from postnatal factors, a higher pre-pregnancy maternal weight and maternal weight gain during pregnancy has been associated with a higher infant weight and fat mass percentage at birth [10, 11]. Given the increasing prevalence of overweight in adult women, including those at reproductive ages [12], this could potentially contribute to a cross-generational vicious obesity circle. However, it is not known whether these maternal factors have a lasting influence on infant adiposity development. Most studies have focused on weight, length, and other anthropometric measures as a proxy for adiposity in infancy, instead of accurate body composition, but with air-displacement plethysmography infants body composition (i.e. fat and fat-free mass) can accurately be measured [13-15].

Total body fat has adverse consequences on adult disease risks, but location of body fat seems to be even more important. Abdominal visceral fat mass during childhood is associated with an unfavorable metabolic profile in later life [16, 17]. Recently, an ultrasound methodology has been validated to measure infant visceral fat and abdominal subcutaneous fat, enabling a non-invasive approach to obtain more insights in the development of these fat depots during infancy [18].

Our aim was to identify the determinants of total fat mass percentage (FM%), as well as visceral and abdominal subcutaneous fat in the first months of life. Our primary hypothesis was that, independent of birth weight, early weight gain between birth and 3 months and more specifically the gain in FM% between 1 and 3 months of age are associated with a higher fat mass and visceral fat mass at 3 and 6

66 months. Our secondary hypothesis was that longer duration of exclusive breastfeeding is associated with
67 more subcutaneous fat. Our third hypothesis was that pre-pregnancy BMI and maternal weight gain
68 during pregnancy ~~would negatively influence~~ are associated with a higher infant FM% and visceral fat
69 mass at 3 and 6 months.
70 We, therefore, measured in a cohort of healthy term infants longitudinal total body composition by air-
71 displacement plethysmography at 1, 3 and 6 months of age as well as visceral and abdominal
72 subcutaneous fat by ultrasound at 3 and 6 months of age and associated these outcomes with infant
73 data, type of feeding and maternal variables.

74

Material and Methods

Subjects

The study population consisted of 300 healthy term infants, who are embedded in a larger birth cohort study (Sophia Pluto Study) aimed at examining the postnatal determinants of body composition during infancy. Infants were recruited from several hospitals in and near Rotterdam, a large city in The Netherlands (600 000 habitants). All participants fulfilled the same inclusion criteria: 1) born at term (≥ 37 weeks of gestation), 2) Age at recruitment < 28 days, 3) uncomplicated neonatal period without severe asphyxia (defined as an Apgar score below three after five minutes), sepsis or long-term complication of respiratory ventilation. Exclusion criteria were known congenital or postnatal diseases that could interfere with body composition development, confirmed intra-uterine infection, maternal use of corticosteroids or a maternal medical condition that could interfere with infant's body composition development (e.g. diabetes). The Medical Ethics Committee of Erasmus Medical Center approved the study. Written informed consent was obtained from both parents unless the mother was single.

Data collection and measurements

Parental and pregnancy characteristics

Maternal data, i.e. pre-pregnancy weight and highest weight in pregnancy, height, parity, smoking, ethnicity and complications during pregnancy, were obtained from medical records and questionnaires. The Institute of Medicine published in 2009 revised gestational weight gain guidelines to minimize the negative health consequences for both mother and fetus of inadequate or excessive gain. They include four classifications of preconception body mass index (BMI; World Health Organization definitions); underweight, normal weight, overweight and obese. Maternal underweight was defined as a pre-pregnancy BMI $< 18.5 \text{ kg/m}^2$, overweight as a BMI $\geq 25 \text{ kg/m}^2$, and obesity as a BMI $\geq 30 \text{ kg/m}^2$. Weight gain recommendations were given per pre-pregnancy BMI-category, include three categories; too less weight gain, normal weight gain and too much weight gain [19].

Information regarding socioeconomic status, educational levels of the parents was obtained using questionnaires.

The Dutch Standard Classification of Education was used to categorize mothers to one of four levels of education: high (university degree), mid-high (higher vocational training, Bachelor's degree), mid-low (> 3

years general secondary school, intermediate vocational training), low (no education, primary school, lower vocational training or 3 years or less general secondary school)[20].

Infant characteristics

Research clinic visits were scheduled at ages 1, 3 and 6 months. Birth data, such as gestational age, were taken from midwife- and hospital records. Information on breast- and formula feeding was asked at the clinic visits.

Anthropometrics

Weight was measured to the nearest gram by an electronic infant scale (Seca, Hanover, MD), length was measured twice in all infants by the same two persons using the two-persons technique to the nearest 0.1 cm by a length measuring board (Seca). In case of >5mm deviation between the 2 measurements an additional measurement was performed and the mean of the measurements closest together were used. Standard deviation (SD) scores for birth length, birth weight, weight, length and weight for length were calculated to correct for gestational age and gender with Growth Analyser Research Calculation Tools 4.0 (available at www.growthanalyser.org), according to Dutch age- and gender-matched reference values [21].

Body composition

Whole-body composition was estimated using air-displacement plethysmography (ADP) using the Peapod, Infant Body Composition System (COSMED) [14, 15, 22, 23]. Briefly, this ADP system assesses fat mass, fat mass percentage (FM%) and fat free mass and fat-free mass percentage by direct measurements of body volume and body mass, based on the whole-body densitometric principle. All measurements were obtained by experienced personnel, according to standardized protocol. The Peapod was calibrated every day, according to the protocol recommended by the supplier.

Abdominal fat

Visceral and abdominal subcutaneous fat were estimated at 3 and 6 months using a Prosound 2 ultrasound (US), with a UST-9137 convex ultrasound transducer (both from Hitachi Aloka Medical, Switzerland). Two experienced researchers performed all US measurements, after an extra training to measure subcutaneous and visceral fat in infants. To assess the intra-observer and inter-observer

repeatability of the measurements, we calculated the intra-class and inter-class correlation coefficients (ICCs). The ICCs ranged from 0.75 to 0.97, indicating that our measurements were reproducible. For both measures, the transducer was positioned where the xiphoid line intercepted the waist circumference measurement plane. Visceral fat was estimated by measuring visceral depth, which is the distance in cm between the peritoneal boundary and the corpus of the lumbar vertebra, assessed in the longitudinal plane with the ultrasound probe depth set at 9 cm. Subcutaneous abdominal fat was estimated by the distance in cm between the cutaneous boundary and the linea alba at the same location, but on a transverse plane with a probe depth of 4 cm [18].

Statistical analysis

The number of infants at birth and at 1, 3 and 6 months is shown in Supplemental Figure 1. Baseline characteristics are expressed as means and standard deviation, as variables were normally distributed. Linear regression analyses were performed to determine associations between birth weight SDS, birth length SDS, change in body fat mass percentage (FM%)(Δ FM%_{1-3mo}) between 1 and 3 months of age, duration of exclusive breastfeeding, maternal pre-pregnancy BMI (as exact value and as category(according to [19])), maternal weight gain during pregnancy (as exact value and as category per pre-pregnancy BMI [19], and the outcome variables: FM%, visceral and abdominal subcutaneous fat at the age of 3 and 6 months, with adjustment for gender and age. Multiple linear regression (MR) analysis was performed to determine which variables contributed to the FM% and visceral and abdominal subcutaneous fat at the age of 6 months. All models were adjusted for birth weight SDS. First, we entered age, gender, duration of exclusive breastfeeding to the model ((Model A). Secondly, we added the change in FM% from age 1 to 3 months (Δ FM%_{1-3mo}) (Model B). Thirdly, we entered the change in weight for length SDS from age 0 to 1 months (Δ W/LSDS_{0-1mo}) instead of the Δ FM%_{1-3mo} (Model C). Finally, the change in weight for length SDS from age 1 to 3 months (Δ W/LSDS_{1-3mo}) was added instead of Δ W/LSDS_{0-1mo} (Model D). SPSS statistical package version 20.0 (SPSS Inc. Chicago, Illinois) was used for analysis. Results were performed two-sided and were regarded statistically significant if p was <0.05.

Results

Clinical characteristics

Maternal and infant demographic characteristics are presented in Table 1, including the numbers of infants at every visit. The mean age of the mothers was 32.3 years and gestational age was 39.7 weeks. Fifty-eight percent of the infants were male and 87% were Caucasian. Mean fat mass percentage (FM%) increased from 16.7% at 1 month to 22.8% at 3 months and 23.8% at 6 months of age, whereas mean visceral fat remained stable between 3 and 6 months of age. At start, 240 infants received exclusive breastfeeding (80%), at 1 month 165 infants (55%), at 3 months 114 infants (38%) received exclusive breastfeeding and at 6 months 57 infants received exclusive breastfeeding. The mean(SD) duration of breastfeeding was 16(12) weeks. Mean(SD) maternal pre-pregnancy BMI was 24.5(4.7) kg/m², maternal weight gain during pregnancy was 14.0(10.4) kg. Of all mothers, 4% had an underweight pre-pregnancy BMI, 61% had a normal pre-pregnancy BMI, 22% an overweight pre-pregnancy BMI and 13% an obese pre-pregnancy BMI. Weight gain during pregnancy differed between maternal pre-pregnancy BMI-category ($p < 0.001$), with a substantially lower weight gain in obese mothers.

Linear correlations with infant FM% at 6 months

Table 2 shows the linear correlations of infant and maternal variables with FM% at 3 and 6 months, corrected for gender and age. Gain in FM% ($\Delta \text{FM\%}_{1-3\text{mo}}$) and in weight for length SDS ($\Delta \text{W/LSDS}_{1-3\text{mo}}$) between 1 and 3 months of life were both positively associated with FM% at 6 months (all $p < 0.001$). Duration of exclusive breastfeeding was positively associated with FM% at 6 months ($p < 0.01$). None of the selected maternal variables including pre-pregnancy BMI and maternal weight gain during pregnancy were associated with FM% at 6 months. Similarly, there was no relation between the different pre-pregnancy BMI categories or weight gain categories per pre-pregnancy BMI and the FM% at 6 months.

Associations with visceral fat at 3 and 6 months

Table 3 shows the linear associations, corrected for gender and age, of infant and maternal variables with visceral and subcutaneous fat at 3 and 6 months.

Visceral fat

Delta FM%_{1-3mo} was positively associated with visceral fat at 3 months ($p=0.02$) and delta FM%_{3-6mo} with visceral fat at 6 months ($p=0.02$). Delta FM%_{1-3mo} was not associated with visceral fat at 6 months ($p=0.99$). Similarly, delta W/LSDS_{1-3mo} was positively associated with visceral fat at 3 months ($p=0.01$), but not at 6 months ($p=0.95$), while the delta W/LSDS_{3-6mo} showed a positive trend with visceral fat at 6 months ($p=0.06$).

Subcutaneous fat

Delta FM%_{1-3mo} was positively associated with subcutaneous fat at 3 and 6 months ($p=0.01$, $p<0.001$, resp.) and delta FM%_{3-6mo} with subcutaneous fat at 6 months ($p<0.001$). Delta W/LSDS_{1-3mo} was not associated with subcutaneous fat at 3 months ($p=0.16$), but both delta W/LSDS_{1-3mo} and delta W/LSDS_{3-6mo} were associated with subcutaneous fat at 6 months (both $p<0.001$).

Duration of exclusive breastfeeding was positively associated with subcutaneous fat at 3 and 6 months ($p=0.01$, $p=0.01$, resp.), but not with visceral fat (Table 3).

Maternal pre-pregnancy BMI was neither associated with infant visceral nor with subcutaneous fat at 3 and 6 months (neither as exact increase nor based on category). Also weight gain during pregnancy was not associated with visceral or subcutaneous fat at 3 and 6 months (neither as exact increase nor based on category). Parity was only positively associated with subcutaneous fat at 6 months ($p=0.02$). Other maternal variables were not associated with visceral or subcutaneous fat.

Determinants of FM%, visceral and subcutaneous fat at 6 months of age

To identify which of the variables were the most important determinants of FM% at 6 months, we performed multiple regression (MR) analyses (Table 4). Model A shows that female gender and duration of exclusive breastfeeding were positively associated with FM% at 6 months ($\beta=2.719$, $p<0.001$ and $\beta=0.130$, $p<0.001$, resp.). The change in FM% between the age of 1 and 3 months (delta FM%_{1-3mo}) was positively associated with FM% at 6 months next to female gender and duration of exclusive breastfeeding (Model B). The change in W/L SDS between birth and 1 month (delta W/LSDS_{0-1mo}) was not associated (Model C), but the change in W/L SDS between 1 and 3 months (delta W/LSDS_{1-3mo}) was

210 positively associated with FM% at 6 months ($\beta=2.249$, $p<0.001$), next to female gender and duration of
211 exclusive breastfeeding (Model D).

212 As the gain in FM% ($\Delta \text{FM\%}_{3-6\text{mo}}$) and gain in weight for length $\Delta \text{W/LSDS}_{3-6\text{mo}}$ between 3 and 6
213 months ($\Delta \text{FM\%}_{3-6\text{mo}}$) were the only determinants of visceral fat at 6 months, we did not investigate
214 MR models for 6 month of age.

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Discussion

In this longitudinal study with detailed body composition data during infancy, we found a strong association of gain in FM% and its proxy weight-for-length SDS in the first 3 months of life with FM% at 6 months. Gain in FM% and in weight-for-length SDS between 1 and 3 months was associated with visceral fat at 3 months and a higher gain in FM% between 3 and 6 months was associated with more visceral fat at 6 months. Exclusive breastfeeding duration was positively associated with FM% and subcutaneous fat at 6 months, but not with visceral fat.

The present study shows that especially the gain in FM% between 1 and 3 months leads to a higher FM% at 6 months and more visceral fat at 3 months. We have previously shown that infants with a higher FM% at 6 months, measured by DXA scan, tended to keep this higher FM% during childhood [24] and other studies showed that visceral fat in early life tends to track into childhood and adulthood [25-27]. Our results show that this might start as early as the first 3 months of life, emphasizing the need to monitor growth of infants closely from birth onwards. Long-term follow up of our study will hopefully reveal the potential long-term effects of the early growth trajectory on the amount and location of adiposity in this population.

To our knowledge, this is the first study investigating the influence of pre-defined determinants of infant FM% as well as visceral and subcutaneous fat during the first 6 months of life. Infant growth velocity in the first 3 months of life, represented by gain in weight-for-length SDS was associated with higher FM% at 6 months. The impact of infancy weight for length SDS on body fat % might mediate its previously reported association with type 2 diabetes and cardiovascular diseases risk [28]. Moreover, these data support our previous findings, showing that young adults at the age of 21 years had higher risk for type 2 diabetes and cardiovascular diseases when they had a higher gain in weight for length SDS in the first 3 months, while after 3 months no associations between adiposity and risk factors at 21 years could be found. Our findings are in line with previous reports [2, 29].

We found no association between total body FM% and visceral fat, which is in line with the finding that in children total body FM% is more associated with subcutaneous fat rather than with visceral fat [30]. Our data show that associations with total FM% cannot be simply extrapolated to visceral fat and underline the need for detailed body composition assessment instead.

In contrast to our expectations, infants who were still exclusively breastfed at 6 months had a higher FM% at 6 months than those being exclusive formula fed. Exclusive breastfeeding during infancy has been associated with a lower risk of childhood obesity [31], but apparently this might not be caused by tracking of FM% from infancy. Interestingly, we found that the higher FM% associated with increased breastfeeding duration, could be primarily explained due to more subcutaneous fat and not to more visceral fat. One could postulate that if this difference in body fat distribution lasts throughout childhood, breastfed infants might have a more beneficial adiposity phenotype with a reduced risk for obesity and adult diseases [32].

Apart from type of milk feeding, other parental and heritable factors might have more impact on the body composition and obesity risk of their infants. Surprisingly, maternal variables, like pre-pregnancy BMI, were not associated with infant FM% at 3 and 6 months. In a previous study, we showed that maternal BMI before pregnancy associates with FM% at birth [33] and also other studies have shown associations between maternal variables and FM% of newborns [10, 34], but apparently this is a transient effect which disappears after the first month of life. Our findings are in line with another study, showing that maternal BMI and weight gain during pregnancy had no influence on FM%, measured by DXA, in infants at 6 months of age [35]. In addition, the current study demonstrates that the association between maternal variables and FM% of the infants as present at birth, disappeared already at the age of 3 months.

In conclusion, our study shows that a higher gain in FM% in the first 3 months of life leads to more FM% at 6 months of age and more visceral fat at 3 months. Similar associations were found with a higher gain in weight for length during the first months of life. These might underlie the reported long-term associations between rapid changes in weight for length SDS in early life and risks for obesity and adult diseases in later life. We also show that the consistently reported association between exclusive breastfeeding and higher total body FM% in mid-infancy appears to be due to higher subcutaneous fat, but not visceral fat.

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Conflict of interest statement

MAB and DA are employees of Nutricia Research. This study was an investigator-initiated study, AHK received an independent research grant by Nutricia Research.

283 **References:**

- 284 1 Breij LM, Kerkhof GF, Hokken-Koelega AC: Accelerated infant weight gain and risk for
285 nonalcoholic fatty liver disease in early adulthood. *J Clin Endocrinol Metab* 2014;99:1189-1195.
- 286 2 Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A: Timing and tempo of first-year rapid
287 growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA*
288 2009;301:2234-2242.
- 289 3 Singhal A, Lucas A: Early origins of cardiovascular disease: is there a unifying hypothesis?
290 *Lancet* 2004;363:1642-1645.
- 291 4 Kerkhof GF, Hokken-Koelega AC: Rate of neonatal weight gain and effects on adult metabolic
292 health. *Nat Rev Endocrinol* 2012;8:689-692.
- 293 5 Shamir R: The Benefits of Breast Feeding. *Nestle Nutr Inst Workshop Ser* 2016;86:67-76.
- 294 6 Anderson AK: Association between Infant Feeding and Early Postpartum Infant Body
295 Composition: A Pilot Prospective Study. *Int J Pediatr* 2009;2009:648091.
- 296 7 De Curtis M, Pieltain C, Studzinski F, Moureau V, Gerard P, Rigo J: Evaluation of weight gain
297 composition during the first 2 months of life in breast- and formula-fed term infants using dual
298 energy X-ray absorptiometry. *Eur J Pediatr* 2001;160:319-320.
- 299 8 Fields DA, Gilchrist JM, Catalano PM, Gianni ML, Roggero PM, Mosca F: Longitudinal body
300 composition data in exclusively breast-fed infants: a multicenter study. *Obesity (Silver Spring)*
301 2011;19:1887-1891.
- 302 9 Gianni ML, Roggero P, Orsi A, Piemontese P, Garbarino F, Bracco B, Garavaglia E, Agosti M,
303 Mosca F: Body composition changes in the first 6 months of life according to method of feeding. *J*
304 *Hum Lact* 2014;30:148-155.
- 305 10 Au CP, Raynes-Greenow CH, Turner RM, Carberry AE, Jeffery H: Fetal and maternal factors
306 associated with neonatal adiposity as measured by air displacement plethysmography: a large
307 cross-sectional study. *Early Hum Dev* 2013;89:839-843.
- 308 11 Hull HR, Dinger MK, Knehans AW, Thompson DM, Fields DA: Impact of maternal body mass
309 index on neonate birthweight and body composition. *Am J Obstet Gynecol* 2008;198:416 e411-
310 416.
- 311 12 Statistiek CBvd: Lengte en gewicht van personen, ondergewicht en overgewicht; vanaf 1981
312 Centraal Bureau voor de Statistiek, 2012, 2012,
- 313 13 Breij LM, Kerkhof GF, De Lucia Rolfe E, Ong KK, Abrahamse-Berkeveld M, Acton D, Hokken-
314 Koelega ACS: Longitudinal fat mass and visceral fat during the first 6 months after birth in healthy
315 infants: support for a critical window for adiposity in early life. *Pediatr Obes* 2015;Under review
- 316 14 Ellis KJ, Yao M, Shypailo RJ, Urlando A, Wong WW, Heird WC: Body-composition assessment in
317 infancy: air-displacement plethysmography compared with a reference 4-compartment model. *Am*
318 *J Clin Nutr* 2007;85:90-95.

319 15 Ma G, Yao M, Liu Y, Lin A, Zou H, Orlando A, Wong WW, Nommsen-Rivers L, Dewey KG:
320 Validation of a new pediatric air-displacement plethysmograph for assessing body composition in
321 infants. *Am J Clin Nutr* 2004;79:653-660.

322 16 Ferreira AP, da Silva Junior JR, Figueiroa JN, Alves JG: Abdominal subcutaneous and visceral
323 fat thickness in newborns: correlation with anthropometric and metabolic profile. *J Perinatol*
324 2014;34:932-935.

325 17 Goran MI, Gower BA: Relation between visceral fat and disease risk in children and adolescents.
326 *Am J Clin Nutr* 1999;70:149S-156S.

327 18 De Lucia Rolfe E, Modi N, Uthaya S, Hughes IA, Dunger DB, Acerini C, Stolk RP, Ong KK:
328 Ultrasound estimates of visceral and subcutaneous-abdominal adipose tissues in infancy. *J Obes*
329 2013;2013:951954.

330 19 Institute of M, National Research Council Committee to Reexamine IOMPWG: 2009

331 20 Standaard Onderwijs Indeling 2006, Centraal Bureau van de Statistiek,

332 21 Schonbeck Y, Talma H, van Dommelen P, Bakker B, Buitendijk SE, Hirasing RA, van Buuren S:
333 Increase in prevalence of overweight in Dutch children and adolescents: a comparison of
334 nationwide growth studies in 1980, 1997 and 2009. *PLoS One* 2011;6:e27608.

335 22 Sainz RD, Orlando A: Evaluation of a new pediatric air-displacement plethysmograph for body-
336 composition assessment by means of chemical analysis of bovine tissue phantoms. *Am J Clin*
337 *Nutr* 2003;77:364-370.

338 23 Orlando A, Dempster P, Aitkens S: A new air displacement plethysmograph for the measurement
339 of body composition in infants. *Pediatr Res* 2003;53:486-492.

340 24 Ay L, Hokken-Koelega AC, Mook-Kanamori DO, Hofman A, Moll HA, Mackenbach JP, Witteman
341 JC, Steegers EA, Jaddoe VW: Tracking and determinants of subcutaneous fat mass in early
342 childhood: the Generation R Study. *Int J Obes (Lond)* 2008;32:1050-1059.

343 25 Gesta S, Tseng YH, Kahn CR: Developmental origin of fat: tracking obesity to its source. *Cell*
344 2007;131:242-256.

345 26 Gishti O, Gaillard R, Durmus B, Abrahamse M, van der Beek EM, Hofman A, Franco OH, de
346 Jonge LL, Jaddoe VW: BMI, total and abdominal fat distribution, and cardiovascular risk factors in
347 school-age children. *Pediatr Res* 2015;77:710-718.

348 27 Liu KH, Chan YL, Chan WB, Kong WL, Kong MO, Chan JC: Sonographic measurement of
349 mesenteric fat thickness is a good correlate with cardiovascular risk factors: comparison with
350 subcutaneous and preperitoneal fat thickness, magnetic resonance imaging and anthropometric
351 indexes. *Int J Obes Relat Metab Disord* 2003;27:1267-1273.

352 28 Kerkhof GF, Leunissen RW, Hokken-Koelega AC: Early origins of the metabolic syndrome: role of
353 small size at birth, early postnatal weight gain, and adult IGF-I. *J Clin Endocrinol Metab*
354 2012;97:2637-2643.

355 29 Eriksson JG: Early growth and coronary heart disease and type 2 diabetes: findings from the
356 Helsinki Birth Cohort Study (HBCS). *Am J Clin Nutr* 2011;94:1799S-1802S.

357 30 Liem ET, De Lucia Rolfe E, L'Abée C, Sauer PJ, Ong KK, Stolk RP: Measuring abdominal
358 adiposity in 6 to 7-year-old children. *Eur J Clin Nutr* 2009;63:835-841.

359 31 Armstrong J, Reilly JJ, Child Health Information T: Breastfeeding and lowering the risk of
360 childhood obesity. *Lancet* 2002;359:2003-2004.

361 32 Booth A, Magnuson A, Foster M: Detrimental and protective fat: body fat distribution and its
362 relation to metabolic disease. *Hormone molecular biology and clinical investigation* 2014;17:13-
363 27.

364 33 Breij LM, Steegers-Theunissen RP, Briceno D, Hokken-Koelega AC: Maternal and Fetal
365 Determinants of Neonatal Body Composition. *Horm Res Paediatr* 2015

366 34 Josefson JL, Hoffmann JA, Metzger BE: Excessive weight gain in women with a normal pre-
367 pregnancy BMI is associated with increased neonatal adiposity. *Pediatr Obes* 2013;8:e33-36.

368 35 Ay L, Van Houten VA, Steegers EA, Hofman A, Witteman JC, Jaddoe VW, Hokken-Koelega AC:
369 Fetal and postnatal growth and body composition at 6 months of age. *J Clin Endocrinol Metab*
370 2009;94:2023-2030.

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373 Table 1. Maternal and infant characteristics

	Mean	SD
Infant characteristics		
Gender (boys) (%)	58	
Mode of delivery (cesarean delivery) (%)	32.3	
Gestational age (weeks)	39.7	1.2
Birth weight SDS	-0.38	1.1
Birth length SDS	-0.23	1.2
At 1 month (n=300)		
Weight SDS	-0.30	1.2
Length SDS	0.03	0.9
Fat mass%	16.7	4.6
At 3 months (n=268)		
Weight SDS	0.47	1.1
Length SDS	0.36	0.9
Fat mass%	22.8	5.1
US-visceral fat (cm)	2.54	0.6
US-abdominal subcutaneous fat (cm)	0.42	0.1
At 6 months (n=248)		
Weight SDS	0.20	1.0
Length SDS	0.23	0.8
Fat mass%	23.8	5.3
US-visceral fat (cm)	2.47	0.6
US-abdominal subcutaneous fat (cm)	0.44	0.1
Duration of breastfeeding (wks)	16	12
Exclusive breastfeeding at 1 month (%)	55	
Exclusive breastfeeding at 3 month (%)	38	
Exclusive breastfeeding at 3 month (%)	19	
Maternal characteristics		
Age (y)	32.3	4.8
Height (cm)	168	6.7
Pre-pregnancy weight (kg)	69.7	13.6
Pre-pregnancy BMI (kg/m ²)	24.5	4.7
Weight gain during pregnancy (kg)	14.0	10.4
Highest weight in pregnancy (kg)	83.8	16.1
Highest BMI in pregnancy (kg/m ²)	29.4	5.6
Weight gain per pre-pregnancy BMI-category*		
Underweight pre-pregnancy BMI(<18.5) (4%)	15.7	4.9
Normal pre-pregnancy BMI (18.5-24.9) (61%)	14.1	8.5
Overweight pre-pregnancy BMI (25.0-30.0) (22%)	17.4	7.8
Obese pre-pregnancy BMI (>30.0) (13%)	7.3	18.5
Smoking during pregnancy (%)	4.7	
Caucasian ethnicity (%)	87	
Educational level (%)		
High	26	
Mid-high	25	
Mid-low	21	

	Low	4
	Other or unknown	23

374

375 *: According to the 2009 IOM guidelines [19]

376

377 Table 2. Associations of FM% at 3 and 6 months with infant and maternal variables

	FM% at 3 months		FM% at 6 months	
	β	p-value	β	p-value
Infant characteristics				
Gestational age (weeks)	-0.67	0.01	-0.40	0.18
Birthweight SDS	0.11	0.71	-0.14	0.68
Birth length SDS	-0.10	0.78	-0.01	0.99
At 1 month				
W/L SDS	1.58	<0.001	0.96	0.02
FM%	0.46	<0.001	0.32	<0.001
Delta W/L SDS _{0-1mo}	1.05	0.08	0.59	0.34
At 3 months				
W/L SDS	2.94	<0.001	2.10	<0.001
FM%			0.72	<0.001
Delta W/L SDS _{1-3mo}	2.86	<0.001	2.53	<0.001
Delta FM% _{1-3mo}	0.53	<0.001	0.40	<0.001
At 6 months				
W/L _{SDS}			2.87	<0.001
Delta W/L SDS _{3-6mo}			1.92	0.002
Delta FM% _{3-6mo}			0.52	<0.001
Duration of exclusive breastfeeding (wks)	0.08	0.02	0.13	<0.01
Maternal variables [^]				
Age (y)	0.07	0.33	0.13	0.10
Height of mother (cm)	-0.06	0.38	-0.03	0.61
Maternal pre-pregnancy BMI (kg/m ²)	-0.15	0.11	-0.10	0.31
Maternal weight gain during pregnancy (kg)	0.01	0.76	0.01	0.83
Parity mother	0.88	0.04	0.55	0.23
Smoking mother during pregnancy (yes/no)	0.87	0.58	1.16	0.49
Ethnicity	0.17	0.06	0.18	0.09
Educational level	0.00	0.36	0.00	0.83

378 Values presented are results of multiple linear regression. β =unstandardized regression coefficient. All models are adjusted for
379 gender and age. [^]=Adjusted for birth weight. W/L_{SDS} = weight for length SDS. Delta W/L_{SDS}= gain in weight for length SDS. Delta
380 FM%=gain in FM%. Significant p-values are indicated in boldface

381

Table 3. Associations of ultrasound measurements at 3 and 6 months with infant and maternal variables.

	At 3 months				At 6 months			
	Visceral fat		Subcutaneous fat		Visceral fat		Subcutaneous fat	
	β	p-value	β	p-value	β	p-value	β	p-value
Infant variables								
Gestational age (weeks)	-0.05	0.13	0.01	0.07	0.00	0.74	0.00	0.68
Birthweight SDS	-0.02	0.67	0.00	0.35	0.04	0.83	0.00	0.84
Birth length SDS	-0.03	0.45	0.01	0.15	0.10	0.01	0.00	0.88
At 1 month								
W/L SDS	0.02	0.76	0.02	0.04	0.03	0.52	0.02	0.05
FM%	-0.01	0.11	0.01	0.01	0.00	0.62	0.00	0.30
Delta W/L SDS _{0-1mo}	-0.13	0.07	0.05	0.002	0.07	0.31	0.00	0.88
At 3 months								
W/L _{SDS}	0.07	0.20	0.04	<0.001	0.04	0.44	0.04	<0.001
FM%	0.00	0.63	0.01	<0.01	0.00	0.76	0.01	<0.001
Delta W/L SDS _{1-3mo}	0.16	0.01	0.02	0.16	0.00	0.95	0.04	<0.001
Delta FM% _{0-1-3mo}	0.02	0.02	0.01	0.01	0.00	0.99	0.01	<0.001
At 6 months								
W/L SDS					0.10	0.02	0.05	<0.001
FM%					0.09	0.09	0.01	<0.001
Delta W/L SDS _{3-6mo}					0.14	0.06	0.04	0.004
Delta FM% _{0-3-6mo}					0.03	0.02	0.01	<0.001
Duration of exclusive breastfeeding (wks)	0.00	0.49	0.00	0.01	0.00	0.74	0.00	0.01
Maternal variables								
Age(y)	0.00	0.91	0.00	0.57	0.02	0.13	0.00	0.06
Height of mother (cm)	-0.01	0.08	0.00	0.64	-0.01	0.19	0.00	0.05
Maternal pre-pregnancy BMI (kg/m ²)	0.01	0.43	0.00	0.13	0.00	0.18	0.00	0.57
Maternal weight gain during pregnancy (kg)	0.01	0.14	0.00	0.46	0.00	0.18	0.00	0.20
Parity mother	-0.02	0.79	0.02	0.15	0.07	0.17	0.02	0.02
Smoking mother during pregnancy (yes/no)	0.30	0.11	-0.07	0.10	0.00	1.00	-0.01	0.78
Ethnicity	0.00	0.78	0.00	0.65	0.02	0.09	0.00	0.17
Educational level	0.00	0.47	0.00	0.42	0.00	0.62	0.00	0.42

Values presented are results of multiple linear regression. β =unstandardized regression coefficient. All models are adjusted for gender and age. W/L_{SDS} = weight for length SDS. Delta

W/L_{SDS}= gain in weight for length SDS. Delta FM%=gain in FM%

Significant p-values are indicated in boldface.

Table 4. Multiple regression for FM% at 6 months

	Model A		Model B		Model C		Model D	
	β	p	β	p	β	p	β	p
Age (months)	1.351	0.499	2.354	0.214	1.973	0.328	1.481	0.444
Gender*	2.719	0.001	3.025	<0.001	2.673	0.001	3.259	<0.001
Duration of breastfeeding (wks)	0.130	<0.001	0.111	0.001	0.148	<0.001	0.128	<0.001
Delta FM% _{1-3mo}			0.459	<0.001				
Delta W/LSDS _{0-1mo}					-0.322	0.521		
Delta W/LSDS _{1-3mo}							2.249	<0.001
Overall p-value	<0.001		<0.001		<0.001		<0.001	
Adjusted R ²	0.166		0.297		0.139		0.243	

*gender:0=boys, 1=girls W/L_{SDS} = weight for length SDS. Delta W/L_{SDS}= gain in weight for length SDS. Delta FM%=gain in FM%. Significant p-values are indicated in boldface. All models were adjusted for birth weight SDS

Supplemental figure 1. Participants flow diagram

